12-18-2013

Report for NMDGF Permit: 3417, 2013

Douglas Tave

NEW MEXICO INTERSTATE STREAM COMMISSION

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December 18, 2013

Re: 2013 Annual Report for New Mexico Department of Game & Fish Scientific Collecting and Educational Purpose Permit 3417 SCI

DGF.permits@state.nm.us.
Permits Program
New Mexico Department of Game and Fish
P.O. Box 25112
Santa Fe, NM 87507

Sir/Madam:

Attached is our 2013 Annual Report for NMDGF Scientific Collecting and Educational Purpose Permit 3417 SCI to culture Rio Grande silvery minnow at the Los Lunas Silvery Minnow.

If you have any questions, please call me or e-mail me.

Thank you,

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2013 ANNUAL REPORT

New Mexico Department of Game & Fish

Scientific Collecting and Educational Purpose Permit 3417 SCI

Submitted to

State of New Mexico
Department of Game & Fish
One Wildlife Way
PO Box 25112
Santa Fe, NM 87504

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December 18, 2013

Section 1. 2013 Activities

This 2013 Annual Report describes activities allowed under our New Mexico Department of Game & Fish (NMDGF) Scientific Collecting and Educational Purpose Permit 3417 SCI, which permits the New Mexico Interstate Stream Commission to culture Rio Grande silvery minnow (*Hybognathus amarus*) at the Los Lunas Silvery Minnow Refugium (Refugium), Los Lunas, NM. The permit was granted on January 1, 2012, and it expires December 31, 2015. This report summarizes fish culture activities conducted during calendar year 2013 at the Refugium.

Permitted activities

The following activities were conducted in 2013 under NMDGF Permit 347 SCI :

> Activity 1. Conduct a captive spawning study in the outdoor refugium, using up to 750 fish; 100 fish may be tagged with PIT tags annually.
>
> Activity 2. Overwintering of fish produced during the 2012 study on the effects of high stocking densities on growth and survival in the C tanks to reassess fish health in spring 2013.
>
> Activity 3. Maintain a display aquarium in the office stocked with up to 10 fish.
>
> Activity 4. Maintain a reference collection of 20 eggs, 20 fry, and 120 fish up to 12 months of age, and 20 fish older than 12 months of age.

In addition, the following activities were conducted under permits held by other agencies or at the request of the USFWS:

> Activity 5. Collect and transport fish for Health Assessment Index at the Southwest Native Aquatic Resources and Recovery Center. This consists of 60 fish plus any for disease diagnosis.
>
> Activity 6. Collect genetic samples on 50 fish (fin clips) for the Middle Rio Grande Endangered Species Collaborative Program Genetics Monitoring Program.

Activity 7. Provide the use of the outdoor refugium for the US Army Corps of Engineers Hydrophone Study under the USACE USFWS Permit (Permit TE-797127).

Activity 1. Conduct a captive spawning study in the outdoor refugium, using up to 750 fish; 100 fish may be tagged with PIT tags

Introduction

This activity is a Middle Rio Grande Endangered Species Collaborative Program and NMISC jointly funded project that was permitted for three years by the Service. Both spawning and subsequent grow-out of Rio Grande silvery minnow YOY occurred in 2013. The spawning and grow-out study precluded propagation of additional Rio Grande silvery minnow in the outdoor refugium in 2013.

Hypotheses-- This study was designed to determine aspects of Rio Grande silvery minnow spawning behavior and how variations in habitat use during spring runoff translate into age-0 recruitment for the species using the outdoor refugium as a model system. The outdoor refugium provides an opportunity for hypotheses testing of spawning behavior of the Rio Grande silvery minnow under a more controlled and observable setting than can be achieved in the Rio Grande itself. This study was designed to test the two following hypotheses:

Spawning

- H_0: Rio Grande silvery minnow spawns in the river channel
- H_1: Rio Grande silvery minnow spawns on inundated riparian habitat features
- H_2: Rio Grande silvery minnow is an opportunistic spawner

Life History

- H_0: Egg and larval drift (with no substantial egg retention) contributes substantially (e.g. >25%) to YOY recruitment
- H_1: Riparian spawning with subsequent egg retention contributes substantially (e.g., >25%) to YOY recruitment

Outdoor Refugium Preparation, Equipment, and Materials

Outdoor refugium configuration for the spawning study--For the spawning portion of the study, the outdoor refugium was configured and monitoring equipment placed as described below and as shown in Figure 1-1. The outdoor refugium is composed of a stream, 5 ponds, overbank areas, and shelves (Tave et al. 2011) (Figure 1-1 and 1-2). Water level is determined by gate height (Tave et al. 2011). A flood is created by manipulation of gate height and pumping rate so that water levels exceeds the stream's capacity and inundates surrounding ponds, overbanks areas, and shelves.

The spawning study's configuration was designed to confine fish to the stream prior to the first flood and restrict fish from entering/exiting any other area (Ponds 1, 3, and 5, overbanks, and shelves) except for two selected ponds (Ponds 2 and 4) during the flood. When the outdoor refugium was dry in November-December, 2012, sand bags levees were placed around the edges of the ponds, shelves, and overbanks at a height to prevent fish from entering these areas from the stream during the spawning portion of the study.

For Ponds 2 and 4, sand bag levees were constructed around their perimeters except for a single 1.1-m-wide opening to connect the stream and each pond at flood stage (Figure 1-1). Fish were only be able enter/exit through these openings, where equipment was placed to monitor fish movement during the flood. This configuration would not allow Rio Grande silvery minnow eggs to passively enter the ponds because once the ponds filled with water during the floods, there was no flow into Ponds 2 and 4.

Two HOBO temperature loggers (Onset Computer Corporation, Pocasset, MA) were placed in the outdoor refugium. One was placed in the stream and the other in Pond 4 to provide temperature profiles of the two habitats throughout the spawning component of the study (Figure 1-1).

A DIDSON sonar camera (Sound Metrics, Bellevue, WA) was placed in Pond 2, with the lens facing toward the entrance/exit between the stream and pond (Figure 1-1). The DIDSON was an electronic visual monitor of fish movement at the entrance/exit to Pond 2 and provided a way to passively observe fish movement.

PIT tag readers were placed over the entrance/exit openings to Ponds 2 and 4 and at two locations in the stream to monitor fish movement (Figure1-1).

Three Moore egg collectors were set in the stream approximately 76 cm in front of the rotating barriers (Figure 1-1) to monitor for drifting eggs in the stream.

Other materials/equipment used--Pumping rates were changed for various components of the study. The gate height was adjusted to create the desired water level conditions and changes in the gate were recorded.

Fish sampling equipment included seines, dip nets and 5-gallon buckets.

Fish measurement equipment included a digital scale and a measuring board.

Rio Grande silvery minnow stocked in outdoor refugium--A total of 750 brood stock, 116 with PIT tags (100 were PIT tagged in 2013), were stocked in the outdoor refugium in February, 2013.

Seven hundred twenty fish (lengths of 62-110 mm TL; Lot 10CSDX; Year Class 2010) were shipped from SNARRC to the Refugium on January 9, 2013. The fish were stocked in the A tank recirculating system in the indoor hatchery. Brood stock used in the 2012 spawning study and held in the indoor refugium over the winter was used to make the total of 750 brood fish for the spawning study. One hundred fish received from SNARRC were PIT tagged on January 29, 2013. Sixteen brood fish from the 2012 study that had been PIT tagged in 2011 or 2012 were used in this study; consequently, a total of 116 PIT tagged fish were stocked into the outdoor refugium. All brood fish received a VIE tag prior to stocking.

The broodstock were harvested from the A and B tanks in the indoor hatchery on February 18, 2013, and stocked in the outdoor refugium. The 750 fish were stocked in the outdoor refugium in February so that they could acclimate to the system and allow for natural gonadal development during the seasonal changes from winter to spring.

Outdoor refugium configuration for grow-out of YOY--At the completion of the spawning study in early June the sand bag levees, PIT tag readers, DIDSON camera, and HOBO temperature readers were removed. In the upper portion of the stream, sand bars were constructed using sandbags. Sand Bars 1 and 2 were constructed (Figure 1-2) but Sand Bars 3 and 4 were not constructed in 2013. With those changes, the outdoor refugium was placed in what is called normal culture conditions (Hutson et al. 2011), and these conditions were maintained until harvest. Fish could occupy all available habitats.

Spawning and Grow-out Methodologies

This section describes the methodologies used to create selected hydrologic conditions within the outdoor refugium; to monitor and record physical conditions; and, monitor fish spawning, movement, use of habitats, and growth.

Hydrology to simulate floods--The outdoor refugium was held dry until February 4, 2013, when it was filled with water from the well and from the Village of Los Lunas municipal water to a gate height of 35.6 cm. Systematic analyses prior to the first year of the spawning study provided the necessary relationships used to predict the hydrologic conditions created at various gate heights and pumping rates. When gate height is under 52.1 cm, only the stream is connected and flowing. When gate height exceeds 52.1 cm, inundation begins.

On four occasions between April 20 and June 3, 2013, gate height was raised and flow rate increased to simulate flooding conditions. On April 20, a flood was created over a 5-h period. Initially, gate height was 35.6 cm and pumping rate was 1,514 L/min. Water was added and pumping rate and gate height (water depth at the outlet) were increased to induce a flood. At flood, gate height was 59.7 cm and pumping rate was 6,201 L/min; this combination inundated Ponds 2 and 4. Flood conditions were maintained until May 2, when water level was dropped over an 11.75-hour period to a gate height 53.3 cm and pumping rate was lowered to 2,839 L/min.

On May 3, gate height was 53.3 cm and pumping rate was lowered to 2,461 L/min. On May 4, gate height was set to 52.7 cm and pumping rate lowered to 1,893 L/min. On May 5, gate height was taken to 52.1 cm and pumping rate stayed at 1,893 L/min. This cut off all water flow to Ponds 2 and 4. On May 6, gate height was taken to 44.5 cm over a 6-h period. On May 7, gate height was returned to 35.6 cm, confining the water to the stream; brood fish that did not leave the ponds when water level was brought back down were confined to the ponds as there was no connection between the ponds and stream.

On May 10, a second flood was created over a 6-h period. Gate height was raised from 35.6 cm to 62.2 cm and pumping rate was increased from 1,893 L/min to 6,095 L/min. This flood was held until May 20, when the gate height was lowered to 50.8 cm and pumping rate remained at 6,095 L/min between May 20 and May 22.

On May 23, a third flood was produced over a 3-h period. During this period, gate height was raised from 50.8 cm to 62.2 cm. Pumping rate went from 5,829 L/min to 6,056 L/min during the 3-h period. The third flood lasted until May 31. Gate height was lowered to 53.3 cm and pumping rate was not lowered.

On June 3, a fourth and final flood was produced. Over a 2-h period, the gate height was increased from 53.3 cm to 62.2 cm; pumping rate was 5,829 throughout the 2-h period.

Normal culture conditions--The spawning study ended on June 9, the refugium was reconfigured as described above, and the system was set to normal culture level on June 10. Gates were set at 57.3 cm and pumping rate was lowered to 3,028 L/min. Normal culture level was maintained for grow-out of YOY until harvest.

Water use--The total volume of water added to the outdoor refugium during the study was 2.913 acre-feet with 2,929,000 L (2.375 ac-ft) of it being well water and 664,108 L (0.538 ac-ft) of dechlorinated Village of Los Lunas municipal water. Municipal water was processed through both a 189 L/min Culligan Hi-Flo 42 Model HRF-30T and a 41.6 L/min Culligan Hi-Flo Model 22 Dechlorinator (Rosemont, IL) before being added to the outdoor refugium. Chlorine concentration was measured whenever municipal water was added to the outdoor refugium; it was always 0 mg/L.

Temperature monitoring with the HOBOs--The HOBOs that were placed in Pond 4 and in the stream continuously monitored temperature during the spawning study. At the end of the spawning portion of the study, the HOBOs were removed.

Monitoring fish movement during flood events--The DIDSON that was located in Pond 2 was monitored twice daily to ensure information was being properly recorded and saved. The PIT tag readers were non-functional due to an unidentified source of electronic noise that interfered with transponder signals.

Monitoring for eggs--The Moore egg collectors were monitored daily from April 20 until June 10. In addition, Ponds 2 and 4 were monitored visually during this period, primarily early in the morning and at sunset, and the area was stirred by hand and dip-netted with an aquarium net prior to the floods being brought down to check for eggs and fry.

Fish growth assessment--Fish were sampled on July 15 (12 fish), August 21 (30 fish), and September 25 (33 fish) to assess growth. Fish were measured to the nearest millimeter (TL) and weighed to the nearest 0.01 g. Fish were harvested October 21-22 (65 fish); all fish were measured to the nearest millimeter and weighed to the nearest 0.01 g.

Water quality--Water quality management was done as described in the original TE Permit application and Supplement 1 to that application and in the TE Permit amendment of February 18, 2011. Water quality was assessed twice daily at five sites: Site B (Pond 1), Site E (Pond 2), Site F (Pond 3), Site H (Pond 4) and Site K (just in front of the rotating barriers) (Figure 1-2). Water quality testing sites A and I were not used in 2013 because data from previous years showed that the measurements were similar to those from other sites and provided no additional information that could be used for management. Dissolved oxygen (DO), temperature, and pH were measured at dawn (ca 0600-0700) and at mid-afternoon (ca 1400-1500) every day. Un-ionized ammonia was measured in the afternoon three days per week. One afternoon per week nitrite, alkalinity, chloride, and turbidity were measured. Hardness was measured once at the beginning of the study. If the bottom was not visible, Secchi disc visibility was measured to the nearest 0.5 cm with a 20-cm Secchi disc each afternoon. DO and temperature were measured with a YSI 550A Dissolved Oxygen meter; pH was measured with a YSI pH 100 meter; all other water quality parameters were measured with a YSI 9500 Photometer (YSI, Inc., Yellow Springs, OH); all were calibrated as described in the instruction manuals.

Water quality was managed and monitored similarly to that described for the 2010 and 2011 grow-out studies (Hutson et al. 2011; Tave and Hutson 2011). The exceptions were fertilization rate and technique and application of agricultural gypsum described individually below.

Fertilization--Six fertilization tanks were used as source tanks for fertilization. Each source tank contained 737 L of well water each fertilized with 25 mL of 11-37-0 liquid fertilizer and 500 g of alfalfa pellets. From March 6 to June 14, one tank was added to the outdoor refugium three times per week (i.e., 3 tanks were added per week). On April 22, May 5, May 23, and June 3, the water from all six source tanks was added to the outdoor refugium in a single application because additional water was added to simulate a flood.

From June 17 to October 11, three fertilization tanks were drained into the outdoor refugium three times per week with the exception of four occasions in which two tanks were applied at a time.

A total of 205 fertilization source tanks were drained into the outdoor refugium over the 2013 season. Total fertilizer added to the outdoor refugium during the study was 5.125 L of 11-37-0 (46.59 L/ha) and 102.5 kg (931.2 kg/ha) alfalfa pellets.

Agricultural gypsum--Because alkalinity is much greater than hardness, afternoon pH can rise above 9 due to photosynthesis (Boyd 1990). Management used in the outdoor refugium to maintain pH within the permitted range (≤ 9) is to add finely ground agricultural gypsum (Boyd 1990; Hutson et al. 2012; Tave and Hutson 2011). A different application technique was used this year to improve distribution of agricultural gypsum. During previous grow-out studies, finely ground agricultural gypsum was added directly to the water. This year, the agricultural gypsum was dissolved in water before it was added. A total of 490 kg (4,457 kg/ha) of agricultural gypsum was applied during 12 applications during the 2013 season.

Fish data analysis--Differences in length and weight among the sampling periods and at harvest were assessed by ANOVA. If there was a significant difference (P = 0.05), differences among treatments were assessed by Duncan's multiple range test (P = 0.05) using SAS® software Version 9.3 of the SAS System for Windows 7 (Cary, NC). A growth curve for the YOY was also determined using SAS 9.3.

Results and Discussion

Spawning--For the second time in this three year study, spawning occurred in response to water level changes (floods), and YOY were produced. It is unknown how many brood stock spawned or how many offspring were produced but at least two separate spawns were produced.

HOBO temperature profiles in Pond 4 and stream during this phase of the study are shown in Figures 1-3 and 1-4, respectively.

Flood One (April 20) Observations-- Brood fish were visually observed entering Ponds 2 and 4 during the first flood on April 20. As soon as water levels rose to inundate Ponds 2 and 4, the fish moved with the water into the Ponds 2 and 4.

The Moore egg collectors were examined daily from April 20 until the spawning study ended on June 9 to check for eggs. During the first period of inundation no other monitoring occurred so we would not disturb the brood fish.

Fish were first observed on the DIDSON entering Pond 2 on April 20. The DIDSON recorded fish movement for the duration of the spawning portion of the study.

No eggs or larval fish were detected.

Flood Two (May 10) Observations-- Brood fish were seen moving into Ponds 2 and 4 during this time, as well as moving throughout the stream.

At sunrise and sunset, Ponds 2 and 4 were sampled using small, fine mesh dip nets to look for eggs and larval fish.

Larval fish were first found on May 22 in Ponds 2 and 4. The brood fish spawned in Ponds 2 and 4 in response to the flood induced on May 10 (second flood). Spawning occurred in the ponds. Eggs were not found in the Moore egg collectors or in the stream, and no eggs were found in the ponds.

Flood Three (May 23) Observations-- Brood fish were seen moving into Ponds 2 and 4 during this time, as well as moving throughout the stream.

At sunrise and sunset, the Ponds 2 and 4 were sampled using small, fine mesh dip nets to look for eggs and larval fish.

Larval fish was observed on June 5 that could be differentiated by size from the larval fish found on May 22. This suggests that a spawn occurred in response to the third flood on May 23. Spawning occurred in the ponds. Eggs were not found in the Moore egg collectors or in the stream, and no eggs were found in the ponds.

After the observations of June 5, we were not able to differentiate fish spawned from the two flood events during monthly sampling or at harvest.

Flood Four (June 3) Observations-- Brood fish were seen moving into Ponds 2 and 4 during this time, as well as moving throughout the stream.

At sunrise and sunset, the Ponds 2 and 4 were sampled using small, fine mesh dip nets to look for eggs and larval fish. Two eggs were found in Pond 4 on June 5. It is not known if these eggs were produced in response to flood 3 or flood 4. Eggs were not found in the Moore egg collectors or in the stream.

Growth of YOY-- Lengths and weights of YOY are shown in Table 1-2. The growth curve is shown in Figure 1-4. Mean harvest length (TL) was 79.7 mm and 3.96 g. Growth at sample period 1 (July 15), 2 (August 21) and 3 (September 25) increased significantly ($P = 0.05$). No growth was measured between samples 3 (September 25) and 4 (October 21-22).

Survival-- Of the 750 brood fish stocked, 254 survived to harvest, a rate of 34% survival.

At least 110 YOY were produced; 65 were harvested in October, 13 were removed as fry and brought into the hatchery for observation, and 33 were removed in September. Seventeen of the 33 removed in September were sent to SNARRC on October 2 (along with brood fish) for the annual HAI. We do not know survival for YOY because the initial number of eggs and larval fish were not known. All eggs (and YOY) were considered to be take for the purposes of this spawning study.

Water quality during grow-out-- Monthly mean ± SD water quality values are shown in Table 1-3. Water quality was in within the permitted ranges throughout the study. Hardness, which was measured once at the beginning of the study, was 65 mg/L.

Behavioral observations-- For the first few days after stocking in February, brood fish were observed in all parts of the stream. After the first few days, they had coalesced into a single school near the base of the stream. When disturbed, they would move a few meters up or downstream, but would always return to that area. Prior to the spawning study in 2011, water velocities were determined throughout the system at the various water depths and pumping rates that would be used during the spawning study. This was done to enable us to know the velocity at a given location without disturbing the fish. The location preferred by the brood fish prior to the flood had a water depth of 35.6 cm and a velocity of 0.12 m/s.

Brood fish responded to the first flood by leaving the stream and moved into Ponds 2 and 4 as they were being inundated. During subsequent floods brood fish were observed in both the stream and Ponds 2 and 4.

Schooling behavior of YOY started shortly after the fish were free swimming. Small schools of ~10-mm fish were observed.

Fish of all ages and sizes were most frequently observed grazing along the bottom. Fish were seldom observed between mid-water and the surface.

After the sand bag levees were removed from around the ponds and the outdoor refugium was changed to normal culture condition, brood fish were observed to remain in the ponds most of the time but also were observed moving throughout the system.

References

Boyd, C.E. 1990. Water Quality in Ponds for Aquaculture. Alabama Agricultural Experiment Station, Auburn University, AL.

Hutson, A.M., L.A., Toya, and D. Tave. 2012. Production of the endangered Rio Grande silvery minnow, *Hybognathus amarus*, in the conservation rearing facility at the Los Lunas Silvery Minnow Refugium. Journal of the World Aquaculture Society 43:84-90.

Tave D., and A.M. Hutson. 2011. 2011 Annual Report: USFWS TE Permit 169770-4. Los Lunas Silvery Minnow Refugium, 1000 Main Street NW, Building H, Los Lunas, NM 87031.

Tave, D., G. Haggerty, C.N. Medley, A.M. Hutson, and K.P. Ferjancic. 2011. Los Lunas silvery minnow refugium: a conservation hatchery. World Aquaculture 42(2):28-34, 67.

Table 1-1. Hydrologic conditions in the outdoor refugium during the spawning component of the study: gate height (cm) is water depth at base of the stream and pumping rate is in L/min. At the end of this spawning study, gate height was reduced to 57.6 cm and pumping rate was reduced to 3,028 L/min, which is considered to be normal culture level for grow- out of YOY.

Date	Gate Height	Pumping Rate	Stream Inundated	Ponds 2 and 4 Inundated	Ponds 2 and 4 Connected to Stream
1-Nov-12	0	0			
4-Feb-13	35.6	1,514	X		
18-Feb-13	35.6	1,514	X		
20-Apr-13	59.7	6,201	X	X	X
2-May-13	53.3	2,839	X	X	X
3-May-13	53.3	2,461	X	X	X
4-May-13	52.7	1,893	X	X	X
5-May-13	52.1	1,893	X	X	
6-May-13	44.5	1,893	X	X	
7-May-13	35.6	1,893	X	X	
10-May-13	62.2	6,095	X	X	X
20-May-13	50.8	6,095	X	X	
23-May-13	62.2	6,095	X	X	X
31-May-13	53.3	6,095	X	X	X
3-Jun-13	62.2	6,095	X	X	X
10-Jun-13	57.6	3,028	X	X	X

Table 1-2. Mean±SD for length and weight of Rio Grande silvery minnow at the three sample periods and at harvest (October 21-22). Means for the harvest data were adjusted, as described in the text. Means followed by different letters were significant at P = 0.05.

Sample/date	N (YOY)	Length (mm)	Weight (g)
July 15	12	48.92±3.03[a]	1.32±0.22[a]
August 21	30	64.97±3.59[b]	2.86±0.45[b]
September 25	33	78.45±3.46[c]	4.05±0.60[c]
October 21-22 (Harvest)	65	79.72±4.61[c]	3.96±0.66[c]

Table 1-3. Monthly means±SD for dissolved oxygen (ppm), temperature (C), pH, un-ionized ammonia (ppm), alkalinity (ppm), nitrite (ppm), turbidity (FTU), and chloride (ppm).

Parameter	June	July	August	September	October
Dissolved oxygen am	6.03±0.73	5.88±0.43	6.16±0.50	6.65±0.78	7.98±0.75
Dissolved oxygen pm	8.66±1.42	8.27±1.25	7.86±1.19	8.95±0.89	9.35±0.66
Temperature am	22.3±1.0	24.33±0.84	23.60±0.68	20.44±2.61	12.67±2.19
Temperature pm	27.0±1.4	28.39±1.07	27.14±0.88	24.33±2.1	18.41±1.97
pH am	8.32±0.22	8.24±0.09	8.17±0.09	8.25±0.13	8.54±0.08
pH pm	8.83±0.15	8.81±0.09	8.76±0.09	8.78±0.12	8.82±0.13
Un-ionized ammonia	0.00±0.00	0.00±0.00	0.00±.0.00	0.00±0.00	0.00±0.01
Alkalinity	145.91±30.38	119.20±13.4	125.50±7.42	150.75±14.44	185.00±15.99
Nitrite	0.01±0.01	0.00±.0.01	0.01±0.01	0.01±0.01	0.01±.0.00
Turbidity	2.3±2.6	0.6±1.3	2.3±2.5	1.6±1.9	0.00±0.00
Chloride	1.22±1.07	0.78±0.53	1.70±0.64	1.41±0.72	0.60±0.97

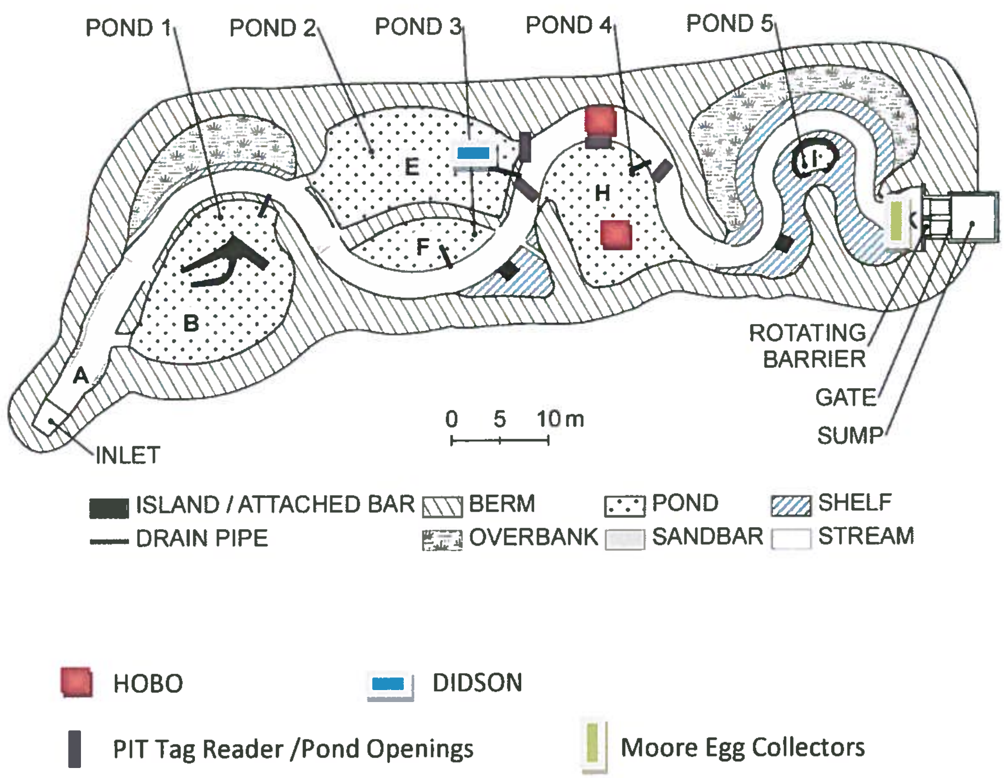

Figure 1-1. Refugium configuration for spawning study.

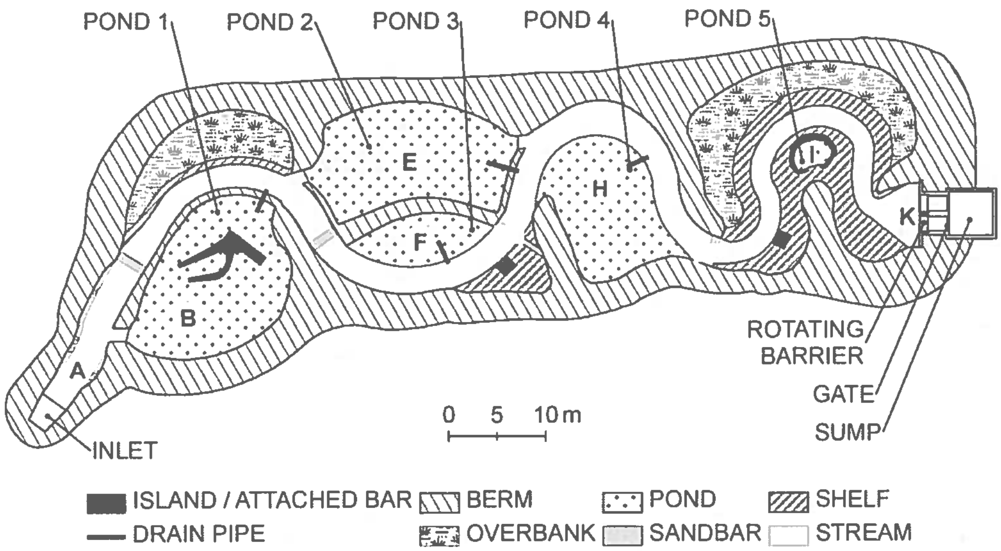

Figure 1-2. Refugium configuration for post-spawning grow-out. Water quality testing sites were B, E, F, H and K.

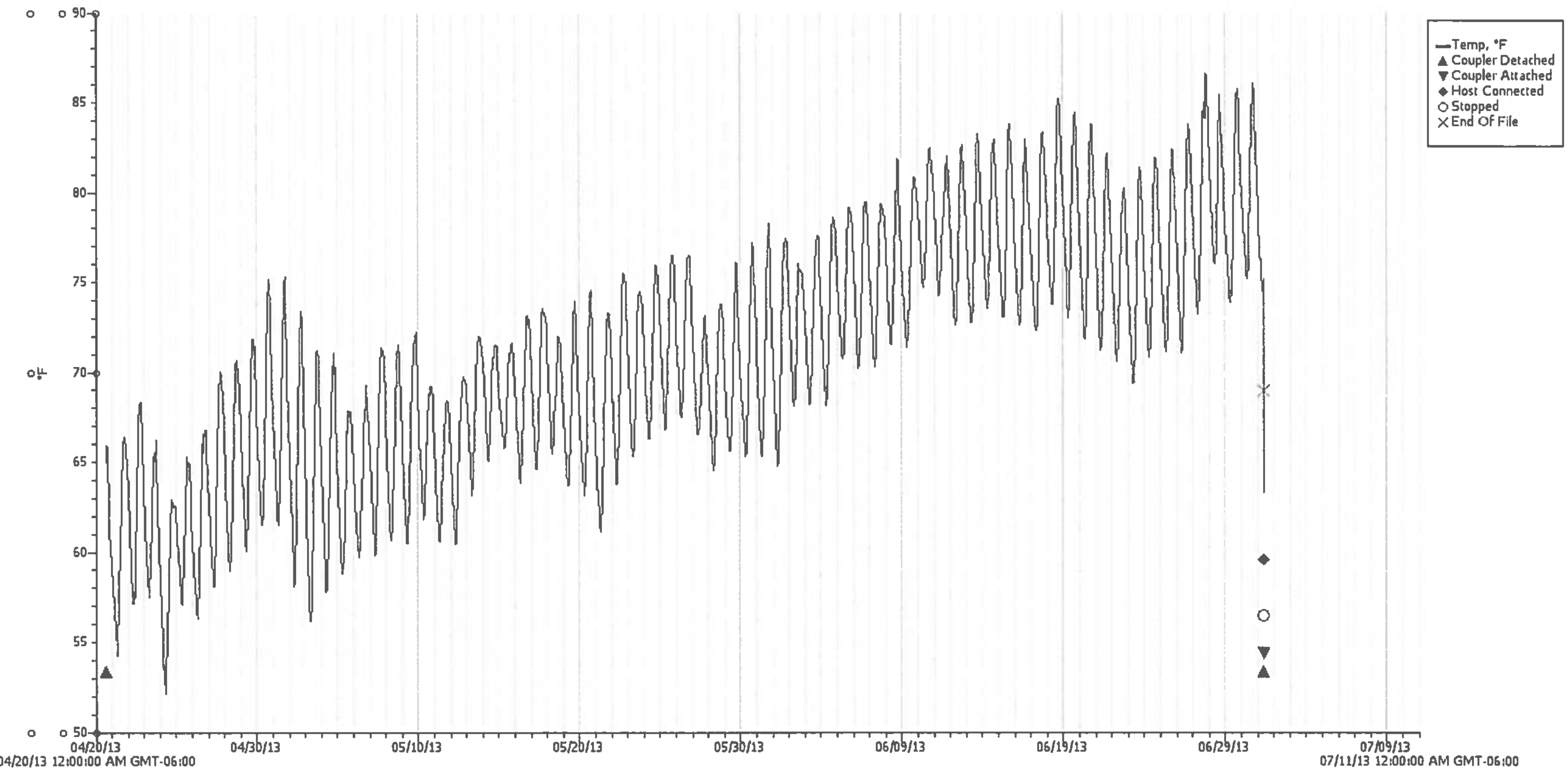

Figure 1-3. Pond temperatures during spawning study.

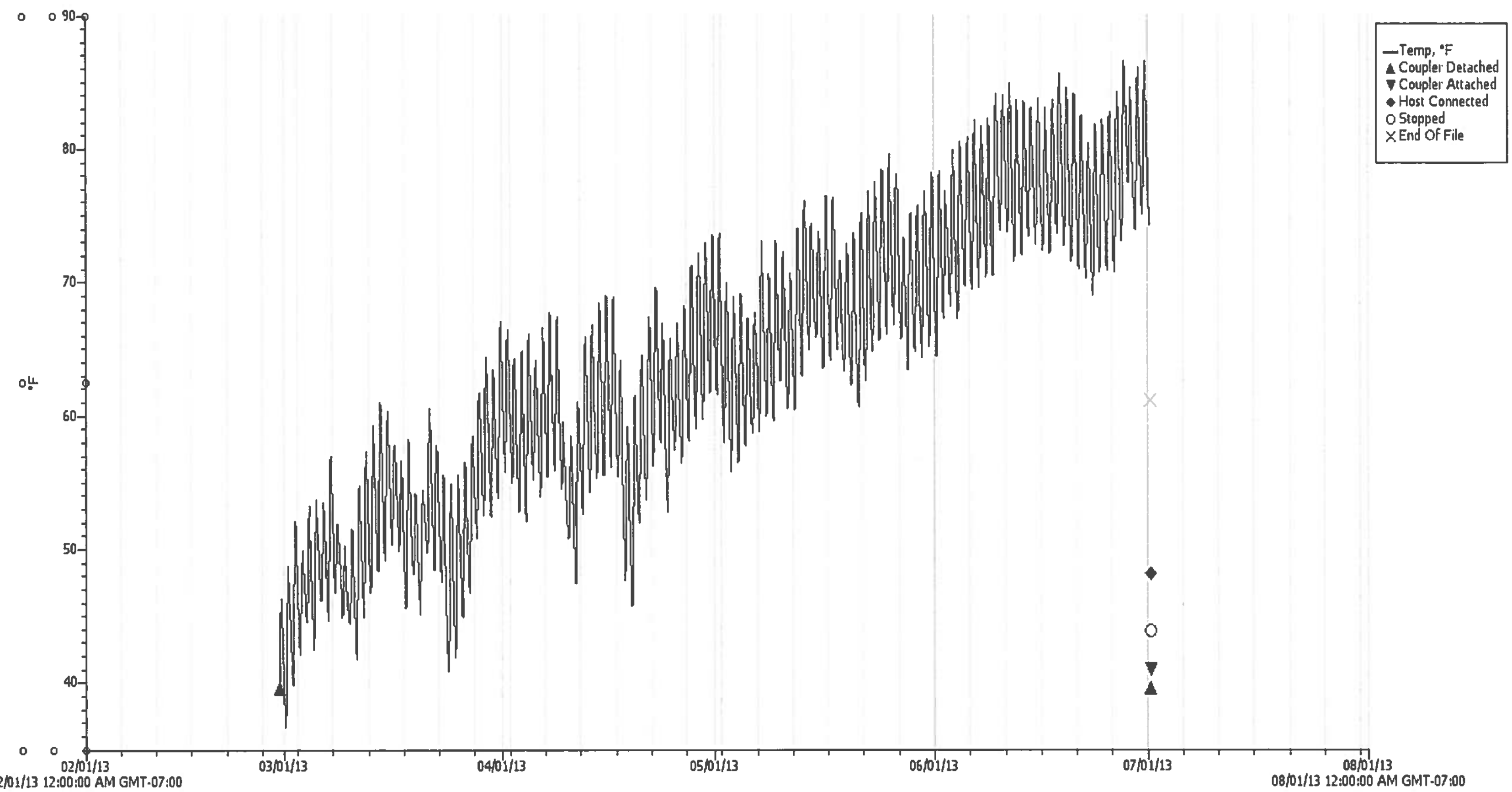

Figure 1-4. Channel temperatures during spawning study.

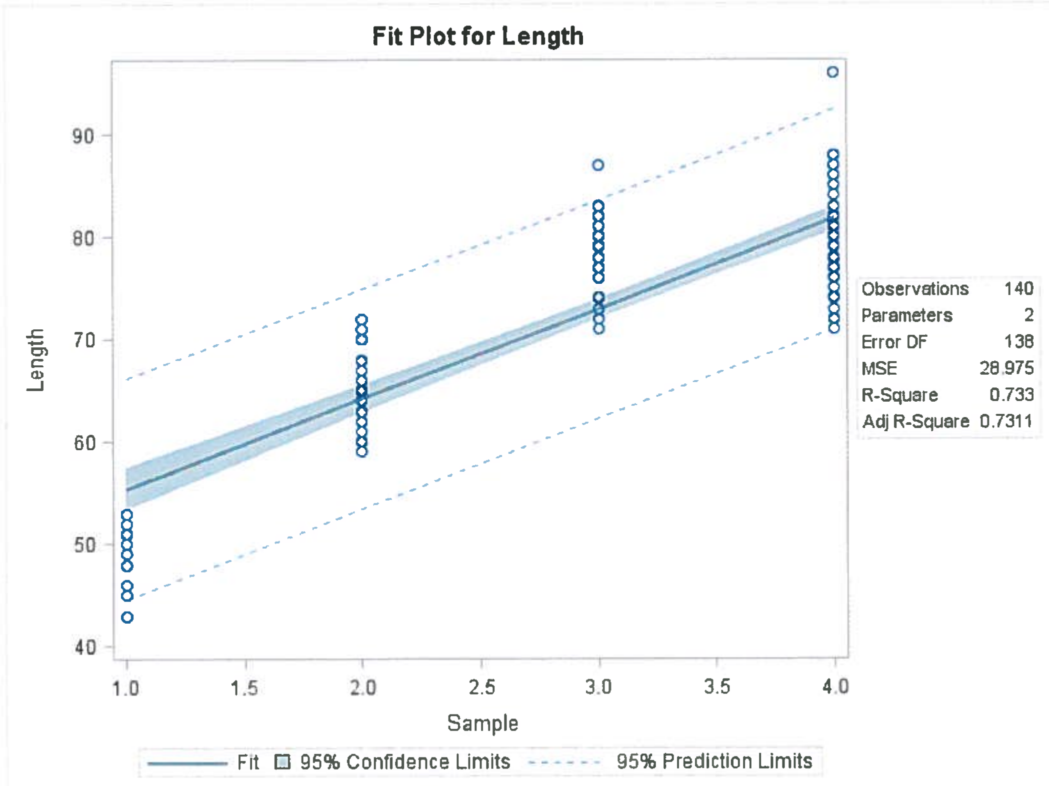

Figure 1-5. Growth curve (total length) of YOY produced during the 2013 spawning study and subsequent grow-out in the outdoor refugium.

Activity 2. Overwintering of fish produced during the 2012 study on the effects of high stocking densities on growth and survival in the C tanks to reassess fish health in spring 2013

Introduction

A yield trial was conducted in the outdoor 2.44-m-diameter above-ground fiberglass C tanks from June 7-October 16, 2012 to determine the effects of high stocking densities (1.07-3.12 million/ha) on growth and survival of Rio Grande silvery minnow. Results of this yield trail were provided in the 2012 Annual Report (Tave and Hutson 2012). In late August, 2012, an unusual behavioral syndrome (spinner) was observed in one of the tanks, and within days it was observed in all the tanks; the syndrome was described in the 2012 annual report (Tave and Hutson 2012). Affected fish were shipped to SNARRC the day that they were observed, but a pathogenic cause could not be determined. Affected fish were also examined by SNARRC personnel at harvest, but a pathogenic cause could not be determined.

These fish were produced for augmentation of the Middle Rio Grande but, because of health concerns, it was decided by Service, in consultation with NMISC hatchery managers, that the fish should not be stocked in the river. Instead, they were restocked into the C tanks after harvest in the fall of 2012 and held in the C tanks during the winter months so that fish health could be reassessed in the spring to determine the fate of the fish.

Materials and Methods

Harvested fish were restocked in six of the outdoor 2.44-m-diameter C tanks October 15-16, 2012. A total of 6,370 fish were restocked; number of fish stocked per tank ranged from 774 to 1,490 (Table 2-1); fish were held in the tanks until March 13, 2013 when they were harvested.

Water used in the study was Village of Los Lunas municipal water that was run through a 189 L/min Culligan Hi-Flo 42 Model HRF-30T Dechlorinator (Culligan International Co., Rosemont, IL, USA). Water depth in the tanks was 78.4 cm; water volume was 3,666 L. Two 14.7- x 3.7-cm air diffusers were placed in each tank to provide continuous aeration. Air supply was provided by an Aquatic Eco-System Sweetwater Model S-51 air blower (Aquatic Eco-Systems, Inc., Apopka, Florida, USA). Tanks were covered with 1.7-cm mesh plastic screens.

Water temperature in the tanks was determined by ambient temperature, and fish were subjected to the daily and seasonal temperature changes that occurred from fall, through winter, and into early spring.

During the 147-day project, temperature, dissolved oxygen (DO), and pH were measured in all tanks in the morning (ca 0700-0800) on 137 days and in mid-afternoon (ca 1400) on 132 days. Un-ionized ammonia was determined 12 times in all tanks. DO and temperature were measured with a YSI 550A Dissolved Oxygen meter; pH was measured with a YSI pH 100 meter; un-ionized ammonia was measured with a YSI 9500 Photometer (YSI, Inc., Yellow Springs, Ohio, USA).

Fish were fed 6 g/tank once weekly from October 15-16 until December 7. Fish were not fed between December 8 and January 27. Fish were fed once weekly from January 28 until harvest.

Fish were harvested March 13, 2013 and enumerated to determine survival.

Results and Discussion

A total of 5,088 fish were harvested; survival was 79.87% (Table 1). Twenty-five spinners were observed at harvest. Because there were still some fish with the behavioral anomaly, Service and NMISC coordinated and decided that the fish should be euthanized and not used for stocking. Service personnel euthanized the fish except for a sample of ten fish that was sent to Dr. Wolfgang K. Vogelbein, Department of Environmental and Aquatic Animal Health, Virginia Institute of Marine Science, The College of William and Mary. He discovered a yet-to-be described amoeba in the brain of the fish (Vogelbein 2013).

Mean±SD morning and afternoon DO were 10.23±3.40 mg/L and 10.21±1.01 mg/L, respectively; mean±SD morning and afternoon pH were 8.55±0.21 and 8.60±0.15, respectively; and un-ionized ammonia was 0 mg/L throughout the study. Temperature during the project is shown in Figure 2-1.

After harvest, the tanks were sterilized by scrubbing them with muriatic acid, rinsing them, scrubbing them with industrial strength Clorox, rinsing them, and then leaving them empty to dry in the direct sunlight during the remainder of 2013. Equipment used in this study was either discarded or sterilized as described above.

References

Tave, D., and A.M. Hutson. 2012. 2012 Annual Report: USFWS TE Permit 169770-5. Los Lunas Silvery Minnow Refugium, 1000 Main Street NW, Building H, Los Lunas, NM.

Vogelbein, W.K. 2013. Histopathological Evaluation of Age-0 Rio Grande Silvery Minnows from the ISC Los Lunas Rio Grande Silvery Minnow Refugium (LLRGSMR). A final Report Submitted to U. S. Fish and Wildlife Service, Region II, Jim Brooks, Project Leader, New Mexico Fish and Wildlife Conservation Office, 3800 Commons Avenue NE, Albuquerque NM. Dept of Environmental and Aquatic Animal Health, Virginia Institute of Marine Science, The College of William and Mary, Rt. 1208, Gloucester Point, VA 23062.

Table 2- 1. Number of Rio Grande silvery minnow stocked per C tank and number harvested in the overwintering project.

Tank	Number stocked	Number harvested	Survival (%)
C1	1,106	635	57.41
C2	774	591	76.36
C3	1,036	879	84.85
C4	1,033	874	84.61
C6	1,490	1,313	88.12
C15	931	796	85.50
Total	6,370	5,088	79.87

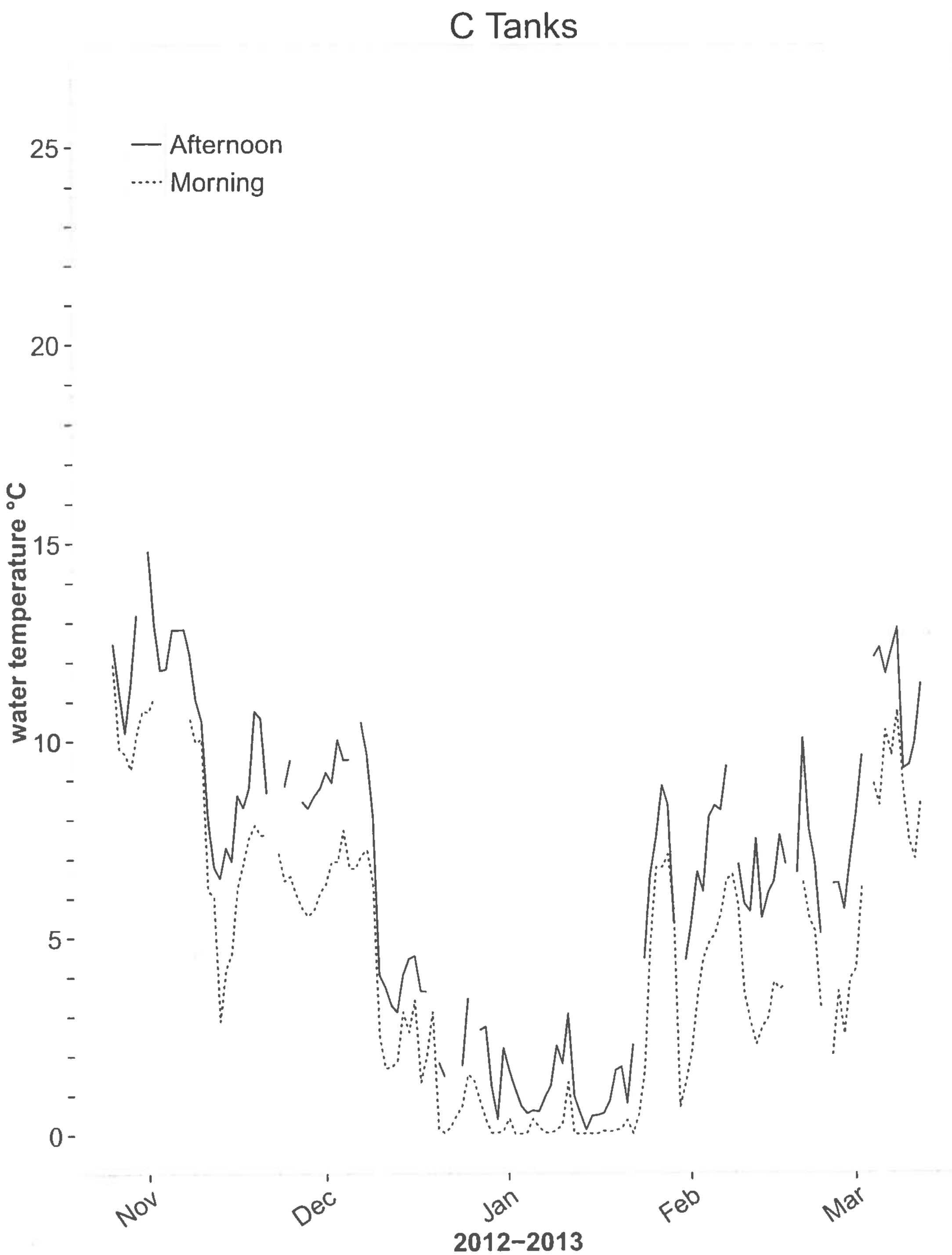

Figure 2-1. Mean morning and afternoon water temperature in the C tanks during the overwintering project. Temperatures were taken in all six tanks.

Activity 3. Maintain a display aquarium in the office stocked with up to 10 fish

The eight fish in the display aquarium are fish that were stocked in 2012. No fish died in 2013, and none were added.

Activity 4. Maintain a reference collection of 20 eggs, 20 fry, and 120 fish up to 12 months of age, and 20 fish older than 12 months of age

Two gravid females that died during the spawning study fish were added to the reference collection in 2013.

The Refugium reference collection consists of eight fry, five 1-month-old fish, five 2-month-old fish, five 3-month-old fish, five 4-month old fish, seven 2-year-old fish, three 3-year-old fish, and two 3-year-old gravid females.

Activity 5. Collect and transport fish for Health Assessment Index at the Southwest Native Aquaculture Resources and Recovery Center. This consists of 60 fish plus any for disease diagnosis

The SNARRC HAI report on 60 fish from removed from the outdoor refugium on September 24-25, delivered to SNARCC personnel on October 3, 2013, is attached. No health problems were detected.

Activity 6. Collect genetic samples on 50 fish (fin clips) for the Middle Rio Grande Collaborative Program Genetics Monitoring program.

Genetics samples from 50 fish collected from the outdoor refugium were taken for the genetics monitoring program on September 24. The fish were a mixed sample of different lots of brood fish and naturally-produced YOY.

Activity 7. Provide the use of the outdoor refugium for the US Army Corps of Engineers Hydrophone Study under the USACE Permit (Permit TE-797127)

The following text was provided by the USACE. The USACE conducted a hydrophone study in the outdoor refugium under the Corps' permit (Permit TE-797127) with concurrence from the NMISC and Service. Hydrophones were deployed in the outdoor refugium prior to stocking with Rio Grande silvery minnow on February 2, and recorded through June 2, 2013. Rio Grande silvery minnow brood fish were stocked on February 18, 2013.

Pre-stocking files provide the baseline (ambient) background sound files for the refugium. Post-stocking files document sounds associated with fish in the facility. Screening of files has identified a suite of sounds for classification and pattern analysis. More than 6,000 audio files were recorded. Sound classification and analysis is pending.

Refugium staff oversaw the placement and operation of the hydrophones. The hydrophones did not interfere with our operations, and no fish were harmed or harassed by the experiment.

Section 2: Update on activities completed in 2012

Food availability-gut study: Resource utilization by Rio Grande silvery minnow in the outdoor refugium in 2012:

Data analysis and final report has not been completed. Data are being analyzed by Dr. Rebecca Bixby at University of New Mexico and Dr. Ayesha Burdett at New Mexico Museum of Natural History and Science. It is anticipated that the final report will be completed in mid-2014. When the final report is accepted as complete by NMISC it will be submitted to NMDGF.

Fatty acid proximate analysis of Rio Grande silvery minnow raised under three types of management, compared to fish from the Rio Grande and to the Rio Grande silvery minnow formulated feed:

Data analysis has not been completed. Data are being analyzed by Drs. Madison Powell and Ronald Hardy at University of Idaho, Hagerman. Results will be included in the 2014 Annual Report.

Section 3: Take during 2013

Brood fish

Post tagging mortality in hatchery prior to stocking: 7

In outdoor refugium during the spawning/grow-out study: 532

Post-harvest in the indoor hatchery: 4

Post-harvest take in the indoor hatchery of YOY prior to augmentation: 3

For health analysis at Southwestern Native Aquatic Resources and Recovery Center: 60

DEPARTMENT OF THE INTERIOR
U.S.Fish and Wildlife Service

FISH HEALTH INSPECTION REPORT[1]

This report is NOT evidence of future disease status. To determine status, contact the inspecting biologist below.

Fish Source & Facility Contact	Fish Examined	Water Supply[2]	5 Year facility classification	
Los Lunas Silvery Minnow Refugium 1000 Main Street, Bldg H Los Lunas, NM 87031 (505)841-5201 Douglas Tave, Ph.D. Manager	☑ Hatchery ☐ Wild	☐ Unsecured: Open Spring, Stream ☒ Secured: Well, Sterilized	Last Sample Date	Classification
			10/03/13	A
			10/16/12	A

Species[3]	Lot Identity	Age[4]	# in lot	(E) Eggs or (F) Fish obtained from	EI	AS	YR	RS	MC	IH	IP	IS	LM	OM	SV	VH	A	B
					Pathogens inspected[3] & results[5] [6]													
RGSM	mixed	mixed		. .	60	60	60			60	60		60		60	60	60	
					-	-	-			-	-		-		-	-	-	
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Remarks[6]: RGSM mixed lots consisted of 10CSDX,2010 WEFO,2011 WEFO and FI, no percentile breakdown. A=Asian Tapeworm.

Inspecting Biologist Signature	Concurred (signature and title)	
Print: Linda Vannest Date: 11/4/2013	Print: Marlene Rodarte Date: 11/4/2013	Dexter Fish Health Unit 7116 Hatchery Road Dexter, NM 88230 (575) 734-5910

[1]Done in accordance with the AFS Fish Health Section Bluebook *Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens* and the U.S. Fish and Wildlife Service Fish Health Policy 713 FW 1-5. [2]Secure = free of all aquatic sterilized. Unsecured = aquatic pathogens may be present.
[3]FWS abbreviations (see back of this page). [4]For hatchery fish give age in months. for feral fish, use symbols: e=eggs or fry; f=fingerling; y=yearlings; b=older fish.
[5] Findings reported as number examined over results; (-) = undetected, (+) = positive, and NT= not tested, A,B = other pathogens as listed in results.
[6]Additional remarks can be made on back page.

DEPARTMENT OF THE INTERIOR
U.S.Fish and Wildlife Service

FISH HEALTH INSPECTION REPORT[1]

Additional Inspection Information
Laboratory Case Number: 14-01

PATHOGEN ABBREVIATIONS

- AS Aeromonas salmonicida
- EI Edwardsiella ictaluri
- RS Renibacterium salmoninarum
- YR Yersinia ruckeri
- MC Myxobolus cerebralis
- IH Infectious Hematopoietic Necrosis Virus
- IP Infectious Pancreatic Necrosis Virus
- IS Infectous Salmon Anemia Virus
- LM Largemouth Bass Virus
- OM Oncorhynchus masou Virus
- SV Spring Viremia Carp Virus
- VH Viral Hemorrhagic Septicemia Virus

SPECIES ABBREVIATIONS

- ALG Alligator gar
- APT Apache trout
- AXR Apache x Rainbow trout
- ARS Arkansas River shiner
- BES Beautiful shiner
- BBG Big Bend gambusia
- BLB Black bullhead
- BLC Black crappie
- BCF Blue catfish
- BLG Bluegill
- BTC Bonytail
- BON Bowfin
- BKS Brook silverside
- BKT Brook trout
- BRB Brown bullhead
- BNT Brown trout
- CCF Channel catfish
- CCH Chihuahua chub
- CCG Clear Creek gambusia
- CPM Colorado pikeminnow
- CSP Comanche Springs pupfish
- CAP Common carp
- CXM Cutbow hybrid
- CUT Cutthroat trout
- DEP Desert pupfish
- DSK Desert sucker
- DHP Devils hole pupfish
- DEV Devils River minnow
- FHM Fathead minnow
- FMS Flannelmouth sucker
- FCF Flathead catfish
- FHC Flathead chub
- FOD Fountain darter
- FRD Freshwater drum
- GIC Gila chub
- GTM Gila topminnow
- GIT Gila trout
- GIS Gizzard shad
- GDE Goldeye
- GOF Goldfish
- GRC Grass carp
- GSF Green sunfish
- GUB Guadalupe bass
- HBC Humpback chub
- KOE Kokanee salmon
- KOI Koi
- LMB Largemouth bass
- LSP Leon Springs pupfish
- LCD Little Colorado spinedace
- LOM Loach minnow
- LSF Longear sunfish
- LFD Longfin dace
- LNG Longnose gar
- MZT Mozambique Tilapia
- SNK Northern snakehead
- PBS Pecos bluntnose shiner
- PAH Paddlefish
- PRC Pahranagat roundtail chub
- PLS Pallid sturgeon
- PEG Pecos gambusia
- PPF Pecos pupfish
- PSS Pumpkinseed
- RBT Rainbow trout
- RBS Razorback sucker
- RES Red shiner
- RDS Readbreast Sunfish
- RSF Redear sunfish
- RGC Rio Grande chub
- RGT Rio Grande cutthroat trout
- RGSM Rio Grande silvery minnow
- RCS River carpsucker
- RKB Rock bass
- RTC Roundtail chub
- WXS Saugeye
- SNG Shortnose gar
- SSN Shortnose sturgeon
- SMB Smallmouth bass
- SAB Smallmouth buffalo
- SDC Speckled dace
- SOS Sonora Sucker
- SPE Spikedace
- SPB Spotted bass
- SPG Spotted gar
- STB Striped bass
- SBH Striped bass hybrid
- TFS Threadfin shad
- VRC Virgin River chub
- WAE Walleye
- WMS Warmouth
- WMF Western mosquitofish
- WHB White bass
- WCF White catfish
- WHC White crappie
- WHS White sucker
- WDF Woundfin
- YCF Yaqui catfish
- YAC Yaqui chub
- YAS Yaqui sucker
- YTM Yaqui topminnow
- YLB Yellow bass
- YEB Yellow bullhead
- YEP Yellow perch